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IN THE U.S. PATENT & TRADEMARK OFFICE

Applicants: Shoji TSUJI et al

Serial No.: 09/101,132 Group: 1635

Filed: June 30, 1998 Examiner: Janet L. Epps

For: cDNA Fragment of Causative Gene of Spinocerebellar Ataxia Type 2

DECLARATION UNDER 37 C.F.R. § 1.132

Honorable Commissioner of Patents and Trademarks

Washington, D.C., 20231

Sir:

I, Hisao UCHIDA, a nation of Japan, residing at 21-4, Shimomeguro 2-chome, Meguro-ku, Tokyo 153-0064 Japan, do hereby declare as follows:

My career is as follows:

1947 Graduated from Department of Chemistry, Faculty of Science, The University of Tokyo

1958 Received Ph, D. degree in Science from The University of Tokyo

1954-1970 Assistant Professor of Institute for Infectious disease, The University of Tokyo

1957-1960 Research Assisstant, Virus Laboratory, University of California, Berkeley

1960-1961 Docent of Max-Planck Inst. for Biology

1970-1986 Professor of The Institute of Medical Science, The University of Tokyo, and Chief of Laboratory of Molecular Genetics thereof

1988 Received Decorated Medal with Purple Ribbon (the highest class of Ribbon Medals presented by Prime Minister)

1986-to date Professor Emeritus, The University of Tokyo

1986-1996 Professor of Teikyo University School of Science and Engineering

Present Adviser of Japan Bioindustry Association

Member of Science Council of Japan

The list of my works (papers) is as follows:

Indole as an activator for in vitro attachment of tail fibers in the assembly of bacteriophage T4D.
Virology. 1969 Jan;37(1):1-7.

Chromosomal mutations affecting the stability of sex-factors in Escherichia coli.
J Mol Biol. 1972 Jan 28;63(2):281-94.

Spectinomycin resistance mutations affecting the stability of sex-factors in Escherichia coli.
J Mol Biol. 1972 Jun 28;67(3):533-5.

Organization and function of bacteriophage T4 tail. I. Isolation of heat-sensitive T4 tail mutants.
Virology. 1973 Mar;52(1):234-45.

Connection of the right-hand terminus of DNA to the proximal end of the tail in bacteriophage lambda.
Virology. 1974 Oct;61(2):524-36.

Late steps in the assembly of 30 S ribosomal proteins in vivo in a spectinomycin-resistant mutant of Escherichia coli.
J Mol Biol. 1975 Aug 15;96(3):443-53.

Organization and function of the tail of bacteriophage T4. II. Structural control of the tail contraction.
J Mol Biol. 1975 Feb 25;92(2):207-23.

Deletion mutations and the replication of DNA in bacteriophage lambda.
Virology. 1977 Dec;83(2):277-86.

Initiation of the DNA replication of bacteriophage lambda in Escherichia coli K12.
J Mol Biol. 1977 Jun 15;113(1):1-25.

Organization and expression of the dnaJ and dnaK genes of Escherichia coli K12.
Mol Gen Genet. 1978 Aug 4;164(1):1-8.

A transducing lambda phage carrying grpE, a bacterial gene necessary for lambda DNA replication, and two ribosomal protein genes, rpsP (S16) and rplS (L19).
Mol Gen Genet. 1978 Oct 24;165(3):247-56.

Genetic analysis of two genes, dnaJ and dnaK, necessary for Escherichia coli and bacteriophage lambda DNA replication.
Mol Gen Genet. 1978 Aug 4;164(1):9-14.

Sigma subunit of Escherichia coli RNA polymerase affects the function of lambda N gene.
Proc Natl Acad Sci U S A. 1979 Sep;76(9):4593-7.

Deletion mapping and heterogenote analysis of a mutation responsible for osmosis-

sensitive growth, spectinomycin resistance, and alteration of cytoplasmic membrane in Escherichia coli.
J Bacteriol. 1980 Aug;143(2):661-7.

Temperature-sensitive mutations in the alpha subunit gene of Escherichia coli RNA polymerase.
J Mol Biol. 1980 Feb 25;137(2):137-50.

Isolation of conditionally lethal amber mutations affecting synthesis of the nusA protein of Escherichia coli.
Mol Gen Genet. 1983;190(2):196-203.

DNA sequencing of the Escherichia coli ribonuclease III gene and its mutations.
Mol Gen Genet. 1985;201(1):25-9.

Suppressors of temperature-sensitive mutations in a ribosomal protein gene, rpsL (S12), of Escherichia coli K12.
Mol Gen Genet. 1985;199(3):381-7.

The Escherichia coli dnaJ mutation affects biosynthesis of specific proteins, including those of the lac operon.
J Bacteriol. 1987 May;169(5):1917-22.

Isolation and characterization of herC, a mutation of Escherichia coli affecting maintenance of ColE1.
Mol Gen Genet. 1989 Nov;219(3):333-40.

Transcription and initiation of ColE1 DNA replication in Escherichia coli K-12. J Bacteriol. 1991 Feb;173(3):1208-14.

I am familiar with the "antisense" technology.

Concerning enablement of claim 5 of the present application directed to antisense nucleic acid, I declare as follows:

Before the priority date (October 30, 1996) of the present application, the antisense technology had been well-known in the art. I will now try to prove this with reference to some references published before the priority date of the present application.

Reference 1 (Nobukuni OGATA et al., Genetic Engineering Keyword Book, published by Yodosha Co. Ltd. on April 25, 1996) is a dictionary carrying the words related to genetic engineering, which is for beginners of genetic engineering. Even such a dictionary for beginners, antisense RNA and antisense DNA are well explained. As described in the item "Antisense RNA", the principle of specific inhibition of expression of a gene by antisense RNA is straightforward. That is, antisense RNA has a nucleotide sequence complementary to a region of the mRNA transcribed from the gene to be blocked. Since an antisense RNA is usually an oligonucleotide having a size of about 20 bp, it can be easily synthesized using a commercial nucleic acid synthesizer. By introducing the antisense RNA into a cell, the antisense RNA hybridizes with the mRNA transcribed from the target gene to be blocked, translation of the mRNA is inhibited, and in turn, expression of the target gene is inhibited. Thus, if the nucleotide sequence of the target gene is known, the gene can be blocked by the antisense RNA. In the present application, since the nucleotide sequence of the SCA2 gene is concretely described in SEQ ID NO:1, it is believed that the antisense RNA can easily be synthesized and expression of the

SCA2 gene can be inhibited by the antisense RNA.

Reference 1 also carries the term "antisense DNA". By inserting the target gene into a vector in the reverse orientation, and by introducing the obtained recombinant gene into a cell, antisense RNA having the nucleotide sequence complementary to the mRNA transcribed from the target gene is produced by transcription from the reversely inserted gene (flipped gene), so that expression of the target gene is inhibited. Since a number of vectors which may be used for introduction of a gene into mammalian cells were known and commercially available, antisense DNAs were also widely used in the art. In the present application, since the nucleotide sequence of the SCA2 gene is concretely described in SEQ ID NO:1, it is believed that the DNA coding for SCA2 can easily be cloned and expression of the SCA2 gene can be easily inhibited by the antisense DNA by the method just mentioned above.

To show how widely the antisense technology is known and used, I would like to refer to several additional references.

Reference 2 (J J Rossi, "Therapeutic antisense and ribozymes", British Medical Bulletin (1995) Vol. 51, No. 1, pp.217-225) describes the use of the antisense technology to therapeutic applications. The preface of Reference 2 reads: "The regulation of expression of genetic information by complementary paring of sense and antisense nucleic acid strands has been termed 'antisense', and is a mechanism used throughout nature to regulate gene expression. **It is now possible to design antisense DNA oligonucleotides, or catalytic antisense RNAs (ribozymes) which can pair with and functionally inhibit the expression of any single stranded nucleic acid in a sequence specific fashion.** This high degree of specificity has made them attractive candidates for therapeutic agents. These molecules have the potential for the treatment of a wide variety of diseases. The recent development of retroviral as well as other viral and non-viral based delivery

schemes make the clinical use of these molecules a virtual certainty." (emphasis added)

Thus, Reference 2 states that expression of inhibition of a gene can be accomplished by the antisense DNA oligonucleotides.

Reference 3 (Saswati Chatterjee et al., "Dual-Target Inhibition of HIV-1 in Vitro by Means of an Adeno-Associated Virus Antisense Vector, SCIENCE VOL. 258, 27 NOVEMBER 1992) describes actual inhibition of HIV-1 by using an adeno-associated virus vector encoding an antisense RNA.

As apparent from the foregoing, and especially in view of the fact that oligonucleic acid was able to be synthesized easily using commercial available nucleic acid synthesizer, and that the sequence of SCA2 is described in the specification of the present application, I believe that the antisense nucleic acid claimed in claim 5 is described in the specification in such a manner that those skilled in the art can easily make and use it.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 16th day of September, 1999

Hisao Uchida

Hisao UCHIDA